

## SEQUENCE LISTING

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CHRISTENSON, STEVEN D.
STANDAGE, SCOTT

- <120> GENE CLUSTER FOR PRODUCTION OF THE ENEDIYNE ANTITUMOR ANTIBIOTIC C-1027
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- <150> 60/115,434
- <151> 1999-01-06
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- <170> PatentIn Ver. 2.1
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agc Ser	atc Ile	gcc Ala	ggc Gly 780	Ala	ctg Leu	cac His	acg Thr	gcc Ala 785	ggt Gly	cag Gln	ctg Leu	gca Ala	ccg Pro 790	ggc Gly	agc Ser	2522
gcc Ala	gac Asp	gcc Ala 795	Leu	ctg Leu	gac Asp	tcc Ser	gcg Ala 800	cgc Arg	gcg Ala	gcc Ala	ttc Phe	acc Thr 805	Ser	ggc	gtg Val	2570

cag tee gte gee gte tge gee gtg tte tee etg geg ete gee gte 2618 Gln Ser Val Ala Ala Val Cys Ala Val Phe Ser Leu Ala Leu Ala Val 810 ctc atc ggc acc cgg ctg cgg gac att tcc gcg atg gac cac ggg cac 2666 Leu Ile Gly Thr Arg Leu Arg Asp Ile Ser Ala Met Asp His Gly His 2715 ggc gag gaa ccg gcc gag aac gac gct caa ccg gcc aca tgagcgcact Gly Glu Glu Pro Ala Glu Asn Asp Ala Gln Pro Ala Thr tccggagatg caacggccgc cgtcgaggta tgaggatcac cttccggggt gcacctgcac 2775 ggcaacggag gcgtagtgga gtactggaac agcacggcgg agaccatgcc ccgccaggaa 2835 ctcgaacagt ggaagtggcg caggctccag gccgccatgg accacgccag aaggctttcg 2895 gtgcctctcc tgcgcaaggc cgacctcctc gccgcggaag ccgcgtctcc cccttacggc 3015 3035 acctggccct cgctggatcc <210> 113 <211> 332 <212> PRT <213> Streptomyces globisporus <220> <223> sgcA <400> 113 Met Arg Met Leu Val Thr Gly Gly Ala Gly Phe Ile Gly Ser Gln Phe Val Arg Ala Thr Leu His Gly Glu Leu Pro Gly Ser Glu Asp Ala Arg Val Thr Val Leu Asp Lys Leu Thr Tyr Ser Gly Asn Pro Ala Asn Leu 35 40 Thr Ser Val Ala Ala His Pro Arg Tyr Thr Phe Val Gln Gly Asp Thr Val Asp Pro Arg Val Val Asp Glu Val Val Ala Gly His Asp Val Ile 70 75 Val His Phe Ala Ala Glu Ser His Val Asp Arg Ser Ile Asp Thr Ala Thr Arg Phe Val Thr Thr Asn Val Leu Gly Thr Gln Thr Leu Leu Glu

110

105

100

Ala Ala Leu Arg His Gly Val Gly Arg Phe Val His Val Ser Thr Asp 115 120 125

Glu Val Tyr Gly Ser Ile Ala Ser Gly Ser Trp Thr Glu Asp Thr Pro 130 135 140

Leu Ala Pro Asn Val Pro Tyr Ala Ala Ser Lys Ala Gly Ser Asp Leu 145 150 155 160

Met Ala Leu Ala Trp His Arg Thr Arg Gly Leu Asp Val Val Thr 165 170 175

Arg Cys Thr Asn Asn Tyr Gly Pro Tyr Gln Tyr Pro Glu Lys Val Ile 180 185 190

Pro Leu Phe Val Thr Asn Ile Leu Asp Gly Leu Arg Val Pro Leu Tyr 195 200 205

Gly Asp Gly Ala His Arg Arg Asp Trp Leu His Val Ser Asp His Cys 210 215 220

Arg Ala Ile Gln Met Val Met Asn Ser Gly Arg Ala Gly Glu Val Tyr 225 230 235 240

His Ile Gly Gly Thr Glu Leu Ser Asn Glu Glu Leu Thr Gly Leu 245 250 255

Leu Leu Thr Ala Cys Gly Thr Asp Trp Ser Cys Val Asp Arg Val Ala 260 265 270

Asp Arg Gln Gly His Asp Arg Arg Tyr Ser Leu Asp Ile Thr Lys Ile 275 280 285

Arg Gln Glu Leu Gly Tyr Glu Pro Leu Val Ala Phe Glu Asp Gly Leu 290 295 300

Ala Ala Thr Val Lys Trp Tyr His Glu Asn Arg Ser Trp Trp Gln Pro 305 310 315 320

Leu Lys Glu Ala Ala Gly Leu Leu Asp Ala Val Gly 325 330

<210> 114

<211> 521

<212> PRT

<213> Streptomyces globisporus

<220>

F F T

<223> sgcB

<400> 114

Met Thr Ala Val Lys Glu Pro Thr Ser Arg Ala Gly Arg Arg Glu Trp
1 5 10 15

Ile Ala Leu Val Val Leu Ser Leu Pro Thr Met Leu Leu Met Leu Asp

30

• • • •

305

Ile Asn Val Leu Met Leu Ala Leu Pro Gln Leu Ser Glu Asp Leu Gly 35 40 Ala Ser Ser Thr Gln Gln Leu Trp Ile Thr Asp Ile Tyr Gly Phe Ala 55 Ile Ala Gly Phe Leu Val Thr Met Gly Thr Leu Gly Asp Arg Ile Gly 70 Arg Arg Arg Leu Leu Gly Gly Ala Ala Val Phe Ala Val Val Ser Val Val Ala Ala Phe Ser Asp Ser Ala Ala Met Leu Val Val Ser Arg 105 Ala Val Leu Gly Val Ala Gly Ala Thr Val Met Pro Ser Thr Leu Ala 120 Leu Ile Ser Asn Met Phe Glu Asp Pro Lys Glu Arg Gly Thr Ala Ile 135 Ala Met Trp Ala Ser Ala Met Met Ala Gly Val Ala Leu Gly Pro Ala 150 155 Val Gly Gly Leu Val Leu Ala Ala Phe Trp Trp Gly Ser Val Phe Leu 165 170 Ile Ala Val Pro Val Met Leu Leu Val Val Thr Gly Pro Val Leu 185 Leu Thr Glu Ser Arg Asp Pro Asp Ala Gly Arg Leu Asp Leu Leu Ser 200 Ala Gly Leu Ser Leu Ala Thr Val Leu Pro Val Ile Tyr Gly Leu Lys 210 215 Glu Leu Ala Arg Thr Gly Trp Asp Pro Leu Ala Ala Gly Ala Val Val Leu Gly Val Ile Phe Gly Ala Leu Phe Val Gln Arg Gln Arg Leu 250 Ala Asp Pro Met Leu Asp Leu Gly Leu Phe Ala Asp Arg Thr Leu Arg 265 Ala Gly Leu Thr Val Ser Leu Val Asn Ala Val Ile Met Gly Gly Thr 280 Gly Leu Met Val Ala Leu Tyr Leu Gln Thr Ile Ala Gly His Ser Pro 290 295

315

Leu Ala Ala Gly Leu Trp Leu Leu Ile Pro Ala Cys Met Leu Val Val

310

Gly Val Gln Leu Ser Asn Leu Leu Ala Gln Arg Met Pro Pro Ser Arg 325 330 335

Val Leu Leu Gly Gly Leu Leu Ile Ala Ala Val Gly Gln Leu Leu Ile 340 345 350

Thr Gln Val Asp Thr Glu Asp Thr Ala Leu Leu Ile Ala Ala Thr Thr 355 360 365

Leu Ile Tyr Phe Gly Ala Ser Pro Val Gly Pro Ile Thr Thr Gly Ala 370 375 380

Ile Met Gly Ala Ala Pro Pro Glu Lys Ala Gly Ala Ala Ser Ser Leu 385 390 395 400

Ser Ala Thr Gly Gly Glu Phe Gly Val Ala Leu Gly Ile Ala Gly Leu 405 410 415

Gly Ser Leu Gly Thr Val Val Tyr Ser Ala Gly Val Glu Val Pro Asp 420 425 430

Ala Ala Gly Pro Ala Asp Ala Asp Ala Gln Glu Ser Ile Ala Gly
435 440 445

Ala Leu His Thr Ala Gly Gln Leu Ala Pro Gly Ser Ala Asp Ala Leu 450 455 460

Leu Asp Ser Ala Arg Ala Ala Phe Thr Ser Gly Val Gln Ser Val Ala

465 470 475 480 Ala Val Cys Ala Val Phe Ser Leu Ala Leu Ala Val Leu Ile Gly Thr 485 490 495

Arg Leu Arg Asp Ile Ser Ala Met Asp His Gly His Gly Glu Glu Pro 500 505 510

Ala Glu Asn Asp Ala Gln Pro Ala Thr 515 520

<210> 115

• 6 j r

<211> 329

<212> PRT

<213> Saccharopolyspora erythraea

<400> 115

Met Arg Val Leu Val Thr Gly Gly Ala Gly Phe Ile Gly Ser His Tyr
1 5 10 15

Val Arg Gln Leu Leu Gly Gly Ala Tyr Pro Ala Phe Ala Gly Ala Asp 20 25 30

Val Val Leu Asp Lys Leu Thr Tyr Ala Gly Asn Glu Glu Asn Leu 35 40 45

Arg Pro Val Ala Asp Asp Pro Arg Phe Arg Phe Val Arg Gly Asp Ile

. . .

Cys Glu Trp Asp Val Val Ser Glu Val Met Arg Glu Val Asp Val Val 65 70 75 80

Val His Phe Ala Ala Glu Thr His Val Asp Arg Ser Ile Leu Gly Ala 85 90 95

Ser Asp Phe Val Val Thr Asn Val Val Gly Thr Asn Thr Leu Leu Gln 100 105 110

Gly Ala Leu Ala Ala Asn Val Ser Lys Phe Val His Val Ser Thr Asp

Glu Val Tyr Gly Thr Ile Glu His Gly Ser Trp Pro Glu Asp His Leu 130 135 140

Leu Glu Pro Asn Ser Pro Tyr Ser Ala Ala Lys Ala Gly Ser Asp Leu 145 150 155 160

Ile Ala Arg Ala Tyr His Arg Thr His Gly Leu Pro Val Cys Ile Thr 165 170 175

Arg Cys Ser Asn Asn Tyr Gly Pro Tyr Gln Phe Pro Glu Lys Val Leu 180 185 190

Pro Leu Phe Ile Thr Asn Leu Met Asp Gly Arg Arg Val Pro Leu Tyr 195 200 205

Gly Asp Gly Leu Asn Val Arg Asp Trp Leu His Val Thr Asp His Cys 210 215 220

Arg Gly Ile Gln Leu Val Ala Glu Ser Gly Arg Ala Gly Glu Ile Tyr 225 230 235 240

Asn Ile Gly Gly Gly Thr Glu Leu Thr Asn Lys Glu Leu Thr Glu Arg 245 250 255

Val Leu Glu Leu Met Gly Gln Asp Trp Ser Met Val Gln Pro Val Thr 260 265 270

Asp Arg Lys Gly His Asp Arg Arg Tyr Ser Val Asp His Thr Lys Ile 275 280 285

Ser Glu Glu Leu Gly Tyr Glu Pro Val Val Pro Phe Glu Arg Gly Leu 290 295 300

Ala Glu Thr Ile Glu Trp Tyr Arg Asp Asn Arg Ala Trp Trp Glu Pro 305 310 315 320

Leu Lys Ser Ala Pro Asp Gly Gly Lys 325 <211> 333

• e , •

<212> PRT

<213> Streptomyces fradiae

<400> 116

Met Arg Val Leu Val Thr Gly Gly Ala Gly Phe Ile Gly Ser His Phe 1 5 10 15

Thr Gly Gln Leu Leu Thr Gly Ala Tyr Pro Asp Leu Gly Ala Thr Arg
20 25 30

Thr Val Val Leu Asp Lys Leu Thr Tyr Ala Gly Asn Pro Ala Asn Leu 35 40 45

Glu His Val Ala Gly His Pro Asp Leu Glu Phe Val Arg Gly Asp Ile 50 55 60

Ala Asp His Gly Trp Trp Arg Arg Leu Met Glu Gly Val Gly Leu Val 65 70 75 80

Val His Phe Ala Ala Glu Ser His Val Asp Arg Ser Ile Glu Ser Ser 85 90 95

Glu Ala Phe Val Arg Thr Asn Val Glu Gly Thr Arg Val Leu Leu Gln
100 105 110

Ala Ala Val Asp Ala Gly Val Gly Arg Phe Val His Ile Ser Thr Asp 115 120 125

Glu Val Tyr Gly Ser Ile Ala Glu Gly Ser Trp Pro Glu Asp His Pro 130 135 140

Leu Ala Leu Ala Tyr His Arg Thr Tyr Gly Leu Asp Val Arg Val Thr 165 \$170 175

Arg Cys Ser Asn Asn Tyr Gly Pro Arg Gln Tyr Pro Glu Lys Ala Val 180 185 190

Pro Leu Phe Thr Thr Asn Leu Leu Asp Gly Leu Pro Val Pro Leu Tyr 195 200 205

Gly Asp Gly Gly Asn Thr Arg Glu Trp Leu His Val Asp Asp His Cys 210 215 220

Arg Gly Val Ala Leu Val Gly Ala Gly Gly Arg Pro Gly Val Ile Tyr 225 230 235 240

Asn Ile Gly Gly Thr Glu Leu Thr Asn Ala Glu Leu Thr Asp Arg 245 250 255

Ile Leu Glu Leu Cys Gly Ala Asp Arg Ser Ala Leu Arg Arg Val Ala 260 265 270

Asp Arg Pro Gly His Asp Arg Arg Tyr Ser Val Asp Thr Thr Lys Ile 275 280 285

Arg Glu Glu Leu Gly Tyr Ala Pro Arg Thr Gly Ile Thr Glu Gly Leu 290 295 300

Ala Gly Thr Val Ala Trp Tyr Arg Asp Asn Arg Ala Trp Trp Glu Pro 305 310 315 320

Leu Lys Arg Ser Pro Gly Gly Arg Glu Leu Glu Arg Ala 325 330

<210> 117

8 (1) p

<211> 331

<212> PRT

<213> Streptomyces argillaceus

<400> 117

Met Thr Thr Thr Ser Ile Leu Val Thr Gly Gly Ala Gly Phe Ile Gly 1 5 10 15

Ser His Tyr Val Arg Thr Leu Leu Gly Pro Arg Gly Val Pro Asp Val 20 25 30

Thr Val Thr Val Leu Asp Lys Leu Thr Tyr Ala Gly Thr Leu Thr Asn 35 40 45

Leu Ala Glu Val Ser Asp Ser Asp Arg Phe Arg Phe Val Arg Gly Asp 50 55 60

Ile Cys Asp Ala Pro Leu Val Asp Asp Leu Leu Ala Val His Asp Gln 65 70 75 80

Ala Ala Asp Phe Val Arg Thr Asn Val Thr Gly Thr Gln Thr Leu Leu 100 105 110

Asp Ala Ala Leu Arg Gln Gly Ile Glu Thr Phe Val His Ile Ser Thr 115 120 125

Asp Glu Val Tyr Gly Ser Ile Asp Ala Gly Ser Trp Pro Glu Thr Ala 130 135 140

Pro Val Ser Pro Asn Ser Leu Tyr Ser Ala Ala Lys Ala Ser Ser Asp 145 150 155 160

Leu Val Ala Leu Ala Tyr His Arg Thr His Gly Leu Asp Val Arg Val
165 170 175

Thr Arg Cys Ser Asn Asn Tyr Gly Ser His Gln Phe Pro Glu Lys Val 180 185 190

Ile Pro Leu Phe Val Thr Ser Leu Leu Asp Gly Arg Glu Val Pro Leu

4 . . . .

195 200 205

Tyr Gly Asp Gly Thr Asn Val Arg Asp Trp Leu His Val Asp Asp His 210 215

Val Arg Ala Ile Glu Leu Val Arg Thr Gly Gly Arg Ala Gly Glu Val 230 235

Tyr Asn Ile Gly Gly Gly Thr Glu Leu Ser Asn Lys Glu Leu Thr Gln 250

Leu Leu Asp Ala Cys Gly Ala Gly Trp Asp Arg Val Arg Tyr Val 260

Thr Asp Arg Lys Gly His Asp Arg Arg Tyr Ser Val Asp Cys Thr Lys 280 285

Ile Arg Arg Glu Leu Gly Tyr Arg Pro Ala Arg Glu Phe Gly Asp Ala 290 295

Leu Ala Glu Thr Val Ala Trp Tyr Arg His His Arg Ala Trp Trp Glu 305

Pro Leu Thr Arg Ala Tyr Gly Ala Val Ala Ala 325

<210> 118

<211> 6

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: 6-His tag

<400> 118

His His His His His